OTKA PD 1121172 project closing report

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The project aimed at studying in detail the effect of red mud (bauxite residue) on soil microflora, flora and fauna, since there are still knowledge gaps on this topic. Our objective was to investigate the changes induced by red mud in sandy soil. The research was conducted in association with the OTKA PD 115871 project.

The first task of the project was the setting up of the soil microcosms during January and February, 2017. Microcosms were set up in triplicate including soil microcosms with red mud (Vi) mixed at 0, 1, 2,5, 5, 7,5 and 10% rate both into the acidic (pH=4.9) sandy soil from Nyírlugos (Nyl) and the calcareous (pH=7,9) sandy soil from Őrbottyán (Őb). Altogether 36 microcosms of 3 kg were prepared, kept at room temperature, wetted every 2 weeks to 60% of their water holding capacity.

The second task was the short- and midterm monitoring of the microcosm experiment, while the fourth task was the long-term monitoring. Samples were taken at the start of the experiment on the day of red mud addition. To study the short term effects, the microcosm was sampled after 2 months, the mid-term effects were monitored after 7 months, the long-term effects after 12 months incubation. The following physical properties were measured: moisture content, water holding capacity, while grain-size distribution of the soils and red mud were measured by microscopy in collaboration with Bay Zoltán Research Institute. The measured chemical properties included: pH, EC, loss on ignition and the total metal content by portable XRF. The available P and K content, N in NO_3^- and NH_4^+ forms, humus content, pH, salt-content, Arany yarn number, total and water soluble toxic element content was measured by ICP-AES at the Research Institute of Soil Science and Agro-chemistry of the Hungarian Academy of Science. We tried to introduce to our laboratory a common spectrophotometric method to measure the amount of water soluble phosphate and nitrite. The microbiological measurements were performed in our research group involving students. We measured dehydrogenase, β-glucosidase, phospho-mono-esterase (PME) and urease enzyme activities, substrate-induced respiration (SIR), microbial community parameters with Biolog EcoPlate (i.e. AWCD, SAWCD, substrate number, Shannon-index), and the total aerobic and anaerobic heterotrophic, fungi, actinoomyceta, N-fixing, phosphate mobilising and oligotrophic (colony forming units) living cell numbers. This complex methodology enabled the assessment of a broad range of microbial effects. To identify the potential environmental risks of red mud application to soil and to assess the effects on soil biota, ecotoxicity tests were performed by students, applying traditional and new endpoints. The following test methods were used: *Aliivibrio fischeri* bioluminescence inhibition test (microtitration plate method applied for the first time to red-mud and soil mixture), *Sinapis alba* and *Triticum aestivum* root and shoot elongation inhibition test, *Tetrahymena pyriformis* reproduction inhibition test, *Dendrobaena veneta*, *Folsomia candida* and *Enchytraeus albidus* avoidance test. Some of the tests were applied not only to whole soil, but also to soil extracts to assess the effects of red mud treatment on groundwater.

The third task was development of new plant and animal ecotoxicity tests. In this respect we tested the Nyl, Őb and a loamy soil from Nagyhörcsök (NH) mixed with 1, 5, 10, 25 and 50% red mud, respectively, applying three plants: common flex, garden cress (*Lepidium sativum*) and Dutch clover. We found that up to 10% red mud addition the garden cress and Dutch clover was not more sensitive than the traditionally used test plants, the white mustard and common wheat. In addition, we faced problems with the germination of common flex thus we did not introduce any new test plant. Simultaneously, we introduced avoidance tests with three animal species: *Dendrobaena veneta* (earthworm), *Folsomia candida* (Collembola) and *Enchytraeus albidus* (enchytraeid worm). In addition to the standard avoidance set up (control-treated), we developed a new, modified test set-up (treated-treated) for the enchytraeid avoidance test. Based on the results of the modified test we concluded that the avoidance (or preference) behaviour, as a sublethal endpoint in this set-up can be successfully used for studying the soil habitat function.

The fifth task of the project was the complex evaluation of the results by statistical methods. We applied ANOVA (Analysis of variance) and correlation analysis for results evaluation. Furthermore, we compared our results (where it was possible) to ideal values for soil based on literature data and Hungarian law. We applied a point system to evaluate the efficiency of the treatments to define the permissible and beneficial red mud amount in the two type of sandy soils.

In summary, we concluded the following:

In terms of toxic metal content of red mud treated soils, 1% and 2.5% red mud addition is acceptable, but above this rate the total arsenic and mobile Mo and V content exceeds the limit value and the Na and Al amount might cause problems. 1% red mud mixed into the originally acidic soil from Nyírlugos resulted in a slightly acidic or neutral pH soil, however starting from 5% red mud addition, the soil became alkaline. The originally alkaline soil from Őrbottyán became strongly alkaline even after 2.5% red mud addition. On the other hand 2.5% red mud into the acidic soil from Nyírlugos improved most of its physical-chemical properties, while it did not show any improvement in the Őrbottyán soil. Red mud mixed into the soil from Nyírlúgos enhanced both on the short and midterm all activities of the microflora (dehydrogenase, SIR, Biolog AWCD, substrate number), the cell number (nitrogen-fixing, phosphate mobilising, aerobic and anaerobic heterotrophic, oligotrophic), however at 10% red mud dose this effect ceased by the 7th months. In general, the cell numbers showed strong correlation with the red mud amount, the pH and the mobile K and P content. It was noteworthy that the activity of β-glucosidase, and the acidic and neutral PME enzymes decreased already upon 1% red mud addition, but the activity of the alkaline PME increased with incremental red mud doses and it continuously increased up to the 7th and 12th month. The PME activities have strongly correlated with the pH and the red mud amount. Several microbiological parameters worsened during the 7 months also in the control soil, which might be attributed to the microcosm as an investigation methodology. This trend attenuated by the 12th month. Interestingly, 1% red mud had positive effect on the activity of the microflora (i.e. Biolog AWCD, SAWCD, β-glucosidase enzyme activity) and cell numbers (nitrogen-fixing,

aerobic and anaerobic heterotrophic, oligotrophic) of the Őrbottyán soils, but higher red mud doses had detrimental effects on soil microbial activity (decreasing the dehydrogenase enzyme activity, SIR, AWCD, SAWCD), thus red mud could not be recommended as soil amendment to calcareous sandy soils. In terms of ecotoxicity red mud was tolerated at up to 5% dose. The plant tests did not indicate toxic effect on the long term, namely after 12 month, however according to the bacterial tests both the untreated (control) and the treated soils were toxic (toxicity decreased with dilution), the protozoa test indicated slight ecotoxicity of the Nyírlugos soil at 7.5% and 10% red mud on the long term, while the soil from Őrbottyán was not toxic. These results are valid both to direct contact soil tests and soil extracts, the latter modelling the effect on ground water.

The avoidance test results after 12 months treatment are the following:

The Collembolas preferred the red mud treated soil from Nyírlugos, while the enchytraeids the Nyírlugos soil treated with more than 2.5% red mud. The earthworms (*Dendrobaena veneta*) did not have any preference. The Collembolas rather avoided the red mud treated soil from Őrbottyán, while the earthworms and enchytraeids had only 1-1 preference, each in this soil. The enchytraeids preferred on the short term the 1–5% red mud treated Őrbottyán soil instead of the control (the untreated). When the animals were offered all the red mud doses paired with all other red mud doses, this newly developed method provided additional information about the soil as habitat for soil biota, compared to the standard avoidance test methods (control and treated soil pair). We developed a new test based on locomotion frequency, showing that the movement of earthworms became slightly stimulated above 2.5% red mud addition to both soils. However, this red mud dose did not stimulate the movement of enchytraeids. In summary, the red mud treated soil from Nyírlugos offered better habitat for soil biota (soil living animals) than the untreated control.

The anticipated results were completed during the project: 1/1. The microcosms were set-up. 1/2. The short and mid-term effects of red mud on soil were investigated through all three sampling periods by a complex methodology. 1/3. We developed new sub-lethal endpoints with three animal test organisms. 2/1. Red mud has changed the soil microbial community composition, stimulated all the microbial activities inhibited the activity of some soil enzymes, while it stimulated the activity of others (alkaline PME). The cell numbers and the microbial activity have decreased with time (but also in the control soil), meanwhile the activity of some enzymes has intensified (the neutral and alkaline PME). The effects depend on the soil pH. 2/2. The red mud had favourable effects on the habitat of the Nyírlugos soil. The enchytraeids preferred the 1–5% red mud treated soil compared to the control. After 12 months the Collembolas and the enchytraeids preferred the red mud treated soil compared to the control. 2/3. Red mud addition has changed also the physical-chemical characteristics of the soil: i.e. pH, grain-size distribution, available P and K, available/mobile toxic metal content. In general, the microbial changes correlated with the red mud dose, soil pH and mobile K and P. The avoidance behaviour of the enchytraeids was dependent on the red mud dose and soil type.

Dissemination: The results of the project was presented in 5 MSc theses and 4 BSc theses, 1 BSc thesis and 1 PhD thesis is under development. A paper earned 2nd place at the Students Scientific Competition of the Faculty and special prize of the American Microbiology Society. Two papers were published in journals with impact factor: the BIOLOG results were presented in the Science of the Total Environment (IF2017=4.610) and the avoidance test results with enchytraeids in Periodica Polytechnica – Chemical Engineering (IF2018=1.382). The results of the project were presented at one international (ICEEM09 2017. Bologna) and four national (KAT17 2017. Debrecen, 7th Conference of the Hungarian Society of Ecotoxicology 2017. Budapest, XIV. Carpathian-basin Environmental Science Conference 2018. Gödöllő, PhD students Conference of the BME VBK ABET 2018. Budapest) conferences. The topic of the project was integrated into teaching (MSc bioengineering). The KÖRINFO/MOKKA database was under development during the project, so no new materials were added there.